ABSTRACT

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The purpose of this study was to investigate the hypothesis that both salinity and low levels of gamma-irradiation effected the osmoregulatory ability of the estuarine grass shrimp, *Palaemonetes pugio*. Groups of grass shrimp were acclimated at three salinity levels and subjected to levels of Co-60 gamma irradiation from 200 to 1800 rads. Changes in the intracellular free amino acid pool were investigated over a period of time after the initial irradiation exposure.

The concentrations of ninhydrin positive substances were shown to vary directly with ambient salinity in *P. pugio*. Non-essential amino acids, glycine, proline and alanine, contributed noticably to the change in the intracellular free amino acid pool. Taurine, a sulfonated amine, is also present in muscle tissue in significant quantities but not directly related to ambient salinity. The effects of radiation exposure on the intracellular free amino acid pool were both time and salinity dependent. This was seen most noticeably in arginine. Although salinity was the overriding effector of changes in intracellular free amino acids, radiation also effects the amounts of free amino acids in the period of time following the initial exposure to irradiation.

THE EFFECTS OF SALINITY AND IRRADIATION ON THE INTRACELLULAR FREE AMINO ACID POOL OF THE GRASS SHRIMP *PALAEMONETES PUGIO*

by

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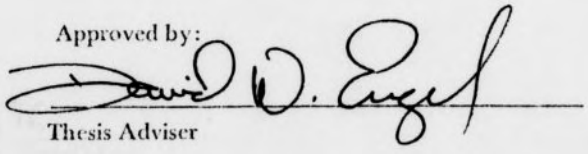

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APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of the Graduate School at the Graduate School at the University of North Carolina at Greensboro.

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INTRODUCTION

The use of nuclear power in the past several decades has increased rapidly. This rapid advance has stimulated interest in the effects of radiation on the aquatic environment. Since a major portion of the estuarine macrofauna is composed of invertebrates, the importance of these species can not be minimized where radiobiological investigations on marine and estuarine organisms are concerned. A number of radiation studies have dealt with the sensitivity of the whole organism to large doses of radiation (Engel, Angelovic, and White 1971; White and Angelovic 1964; Rees 1962) and some studies have dealt with the effects of irradiation on the internal mechanisms of the organism (Conte 1964, Rust et al. 1963, Richardson and Myser 1973).

The estuarine grass shrimp, *Palaemonetes pugio*, has been shown to be extremely sensitive to gamma irradiation (Engel et al. 1971). Studies have shown *P. pugio* to have a 40-day LD-50 of 215 rads which is 5 to 20 times more sensitive than other decapod crustaceans (Engel, Shelton and White 1974; Rees 1962). These LD-50 investigations have also demonstrated that salinity influences the radiation sensitivity of the organism (Angelovic and White unpublished). Since the Palaemonidae are active hypo-hyperosmoregulators (Potts and Parry 1964), the sensitivity to radiation may possibly be correlated with the osmoregulatory processes.

Physiological and ecological studies by Wood (1968) characterized *Palaemonetes pugio* as a euryhaline caridian decapod with an optimum salinity and temperature range of 4-16 ppt and 18° to 25° C, respectively.

Cryostatic investigations by Angelovic and Lewis (unpub.) found *P. pugio* to be isotonic at salinities between 15 and 25 ppt. The ecological habitat of *P. pugio* described by Holthius (1952), Knowlton and Williams (1970) and Williams (1971) reflects a wide salinity tolerance from fresh to sea water. Panikkar (1941) in studying members of the Palaemonidae, *Palaemonetes virians*, *Leander elegans*, and *L. serratus* characterized them as hyperosmotic in low salinity waters and hypoosomotic at high salinities. Odum (1971) described *Paloemonetes* as opportunistic omnivores consuming inorganic material, bacteria, algae or animal material as it was available. *Palaemonetes* are important in some food chains (Odum 1971).

In many euryhaline estuarine invertebrates and especially decapod crustaceans there are two mechanisms of osmoregulation: an anisosmotic regulation involved in regulation of the blood and an intracellular isosmotic regulation in the tissues (Florkin 1962). Anisosmotic regulation involves an ability to actively regulate the ions of the blood plasma in relation to the environment. It has been demonstrated that certain Arthropoda can maintain the blood hypertonic in dilute media or hypotonic in concentrated media. A component of the isosmotic regulatory process is the differential maintenance of levels of free amino acids in the cells. This concept was first postulated by Frederique (1901) and then by Camien et al. (1951). The levels of certain amino acids have been shown to vary directly with the change in ambient salinity. Schoffeneils (1960) demonstrated that the amino acids which act as osmotic effectors are synthesized de novo by the transamination of metabolic intermediates. The synthetic and catabolic pathways involved in the regulation of intracellular free amino acid pools have been extensively investigated in

marine crustaceans (Gilles 1970, Gilles and Schoffeneils 1969a).

The purpose of the investigation was to test the hypothesis that irradiation affects the osmoregulatory ability of *P. pugio*, and therefore may contribute to the radiosensitivity of the organism. To determine if ionizing radiation would affect levels of intracellular amino acids, grass shrimp, *Palaemonetes pugio*, were exposed to different combinations of salinity and radiation, and free amino acid concentrations in muscle were determined at specified times after irradiation. Changes in total and individual amino acid concentrations were compared through time and between salinities and radiation doses.

MATERIALS AND METHODS

The grass shrimp, *P. pugio*, were collected in the Newport River Estuary north of Beaufort, North Carolina where they are abundant throughout the year. At the collection site the salinity varies from 1 ppt to 28 ppt. Collections were made with long-handled dip-nets during low tide when the shrimp were concentrated in the shallow marsh channels.

In the laboratory the nonovigerous adult shrimp were divided into three groups and acclimated to 5, 20, and 35 ppt at 22 ± 2°C, conditions similar to which the shrimp are found in nature. Sea water was prepared by diluting commercial sea salts with distilled water. Salinity was measured with an electrodeless Induction Salinometer with an accuracy of ± 2 ppt. Shrimp acclimated to the higher salinities were brought up gradually from the low salinities at an increase of 10 ppt every two days. All groups were acclimated for one week at test salinities before experiments. The shrimp were maintained in aerated tanks, fed chopped clam daily, and had their water changed ever other day.

After acclimation, the shrimp were irradiated with a single dose of either 200, 600, or 1800 rads. The source of gamma irradiation was a Co-60 Whole Animal Irradiator (ICN, Model 109), which delivered a dose rate of 153 rads per minute ± 10%. The shrimp were irradiated in an aerated 6x6x12 inch lucite irradiation container at the same salinity and temperature to which they were acclimated. After irradiation, the water was changed and the shrimp were returned to aerated 4-liter plastic containers with approximately 20 animals per container. Control animals

were acclimated and treated the same as the test animals except that they were not subjected to irradiation.

Abdominal muscle samples were taken 1, 5, 10, 20, and 30 days after exposure to irradiation. On these days five animals from groups of each radiation-salinity group were selected for tissue removal. Unirradiated animals were taken several times while irradiated animals were being sampled and used as controls. The muscle tissue samples were obtained by first separating the abdomen from the thorax. The carapace was removed with care being taken to separate the ventral nerve and the dorsal vein from the muscle. The tissue was blotted on filter paper to remove extracellular fluids, weighed on a Roller-Smith Precision Balance and extracted in cold (4° C) 5% trichloroacetic acid (TCA) solution. The tissue was then thoroughly mascerated to insure complete percipitation of proteins and extraction of the intracellular free amino acids. The samples were allowed to extract for at least one week at 4° C, centrifuged at 12,000 x g for 15 minutes, and aliquots of the supernate removed for determination of ninhydrin positive substances (NPS), and free amino acids (FAA).

In order to analyze the total intracellular free amino nitrogen pool, NPS were determined. The individual free amino acids found intracellularly were analyzed quantitatively and qualitatively. Total NPS gives an estimation of total free amino nitrogen compounds which include the amino acids as well as trimethylamine oxide, ammonia, and smaller polypeptides. TCA extracts were centrifuged at 12,000 x g for 15 minutes and alliquots of the cold TCA soluable fraction were removed and dilluted with 5% TCA. Then, 0.1 ml of the diluted extract was

analyzed for NPS using the method of Lee and Takahashi (1966) (0.2 ml 0.5M sodium citrate 5.5 pH buffer, 0.5 ml 1% ninhydrin in buffer, 1.2 ml glycerol). This mixture was heated in a water bath to 100° C for 12 minutes in 4 ml Coleman cuvettes. A set of glycine standards were run concurrently with each sample group. Glycine was chosen as a standard because it was the most abundant free amino acid in *P. pugio* samples. After the samples had reacted with the ninhydrin, optical absorbance was measured at 570 nm in a Coleman 6A spectrophotometer.

The separation and quantification of individual free amino acids was done by ion exchange chromatography with a Beckman Model 117 Automatic Amino Acid Analyzer. Cold TCA soluable supernate was dilluted with sodium citrate buffer pH 2.2 containing norleucine as an internal standard. Free amino acids were measured for each control animal but due to the large number of irradiated animals, pooled samples (five animals per sample) were used.

The amino acid analyzer utilizes a modified Moore and Stein (1948) technique for determining amino acids. Acidic and neutral amino acids were separated with Beckman resin AA-15 and 0.2N sodium citrate buffers pH 3.28, and 4.20; the basic amino acids with PA-35 resin and 0.2N sodium citrate buffer pH 5.25. The auto analyzer sequentially prints two absorbance peaks, at 440 and 570nm, which are compared to peaks of Beckman standard amino acids of known concentrations. Amino acids were calculated in μM amino acid per gram wet weight tissue.

RESULTS AND DISCUSSION

Effects of Salinity

The concentrations of ninhydrin positive substances and free amino acids in muscle of unirradiated grass shrimp were related directly to salinity (Figure 1). The high degree of correlation between NPS and FAA values demonstrates that the concentrations of ammonia and other intracellular amines which are not included in the free amino acid total are relatively low in the tissue but do have some contribution to the slightly higher NPS values. NPS and total FAA values increased with an increase in external salinity from 5 ppt to 35 ppt. However, NPS and FAA values at 35 ppt were relatively high when compared with those at 5 ppt and 20 ppt. Shrimp maintained at 20 ppt and NPS values 10% higher than those at 5 ppt whereas those at 35 ppt were 25% higher than the shrimp at 20 ppt which may indicate that the shrimp regulates more stringently at low salinity than at high salinity.

The non-essential amino acids contributed most noticably to the intracellular free amino acid pool in response to changes in the external medium (Table 1). Non-essential amino acids are those amino acids which can be synthesized by the organism: glycine, proline, serine, alanine, aspartic acid, glutamic acid and tyrosine. Glycine, alanine, and proline comprised approximately 70% of the total FAA. Glycine was the most abundant, contributing approximately 40% of the total FAA pool.

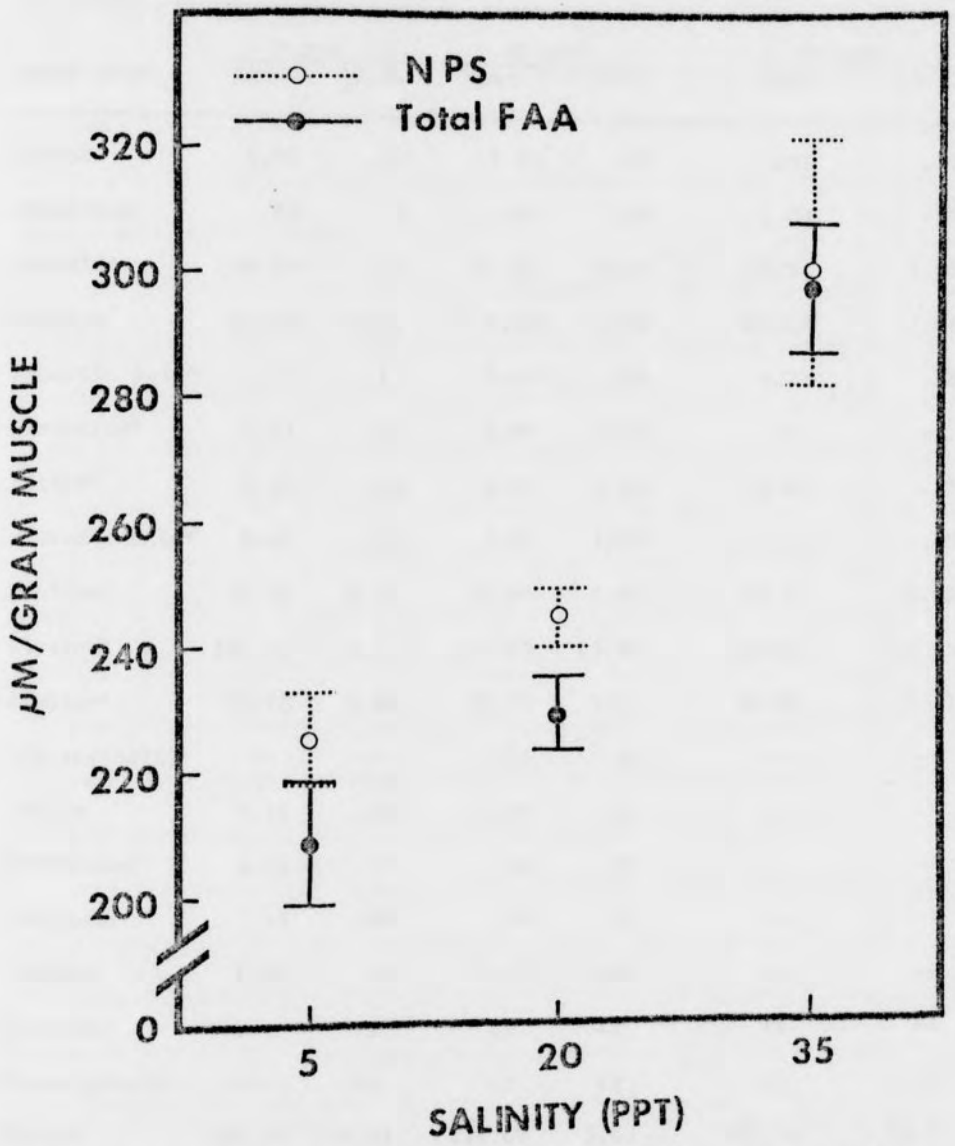

Figure 1. Intracellular ninhydrin positive substances and total free amino acids in muscle tissue of *Palaemonetes pugio*. Vertical bars represent range of standard error.

Table 1.--Concentration of muscle free amino acids in unirradiated *Palaemonetes pugio* (in μM amino acid/gram tissue).

Amino Acid	5 ppt Mean	5 ppt ±S.E.	20 ppt Mean	20 ppt ±S.E.	35 ppt Mean	35 ppt ±S.E.
Lysine	1.40	.33	3.25	.98	.92	.31
Histidine	.93	.12	1.90	.26	1.03	.19
Arginine	24.99	.73	35.51	2.24	38.51	1.95
Taurine	29.33	6.81	8.10	1.40	44.67	7.06
Aspartic Acid*	.98	.17	1.63	.26	1.23	.38
Threonine*	4.91	.65	6.08	1.48	--	--
Serine*	3.59	.80	6.03	2.07	4.88	.24
Glutamic Acid*	6.18	.73	8.37	1.02	5.11	.85
Proline*	19.96	6.74	31.81	6.92	44.67	3.91
Glycine*	105.59	6.11	109.85	13.96	138.36	12.35
Alanine*	13.28	1.69	20.20	3.65	16.94	7.31
Cystine(Half)*	--	--	1.14	.16	--	--
Valine	2.15	.31	3.32	.67	--	--
Methionine	1.25	.17	1.42	.14	--	--
Isoleucine	.39	.06	.94	.17	--	--
Leucine	1.79	.19	2.57	.62	--	--
Tyrosine	tr	tr	tr	tr	tr	tr
Phenylalanine	tr	tr	tr	tr	tr	tr
TOTALS	208.96	10.01	229.64	5.83	296.34	10.25

(tr) = trace; (--) = none detected; * nonessential amino acids

The concentrations of non-essential amino acids varied in relation to each other. The pathways for synthesis of the non-essential amino acids involve many interconnecting metabolic pathways associated with glycolysis and the TCA cycle. While the amounts of amino acid in *P. pugio* muscle showed a certain amount of individual variation at a given salinity, the overall osmotic contribution of the free amino acid pool remained relatively constant.

In considering the amounts of the amino acids, it is important to consider their relative concentrations in relation to each other. In *P. pugio* at all salinities the nonessential amino acids, glycine, proline, and serine showed considerable variation with a net effect of an increase with an increase in salinity (Table 1). Figure 2 illustrates the general relationship of the free amino acids to the TCA cycle. For example, serine is a precursor of glycine: serine can be converted by the organism to glycine and tetrahydrofolic acid with the synthetic reaction predominating (Florkin and Mason 1962). A transamination from glutamic acid yields 3-phosphoserine which undergoes a hydrolysis to yield free serine. Proline is also synthesized from glutamic acid.

Proline, one of the main osmotic effectors in *P. pugio*, was seen to vary directly with the osmotic pressure of the medium. Transmamination of α-ketoglutaric acid yields L-glutamic acid which in turn leads to the biosynthesis of proline. A biodegradation of proline leads to glutamic acid (Awapara and Simpson 1967). Schoffeniels (1970) suggests that the intracellular concentration of proline seems easily controlled through release to the extracellular fluid or by promoting its synthesis intracellularly.

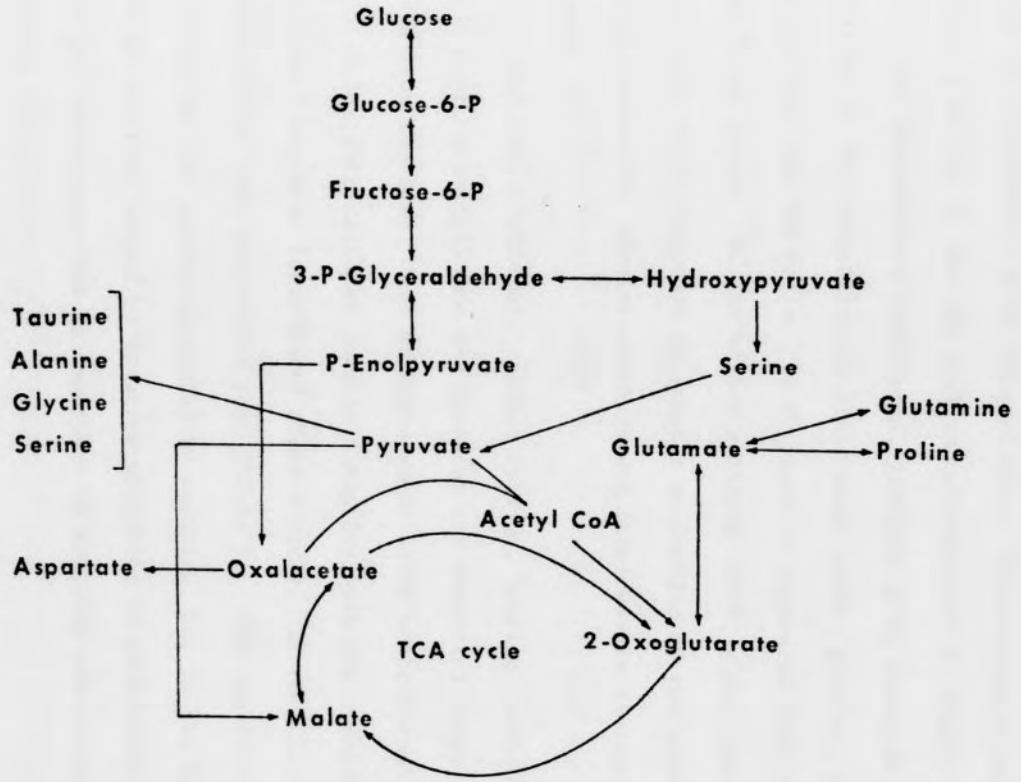

Figure 2. Pathways of carbon carbohydrate incorporation into amino acids in Arthropoda. Adapted from E. Schoffeniels and R. Gilles. 1970. Ch. 7 *in* M. Florkin and B. T. Sheer, eds. Chemical zoology vol. 5. Academic Press, New York and London.

Alanine and aspartic acid occur intracellularly in lesser amounts in *P. pugio*. Alanine and aspartic acid have a metabolic relationship involving synthesis from glutamic acid by its transamination of pyruvic acid and oxaloacetic acid, respectively. Alanine comprised approximately 6 to 10% of the FAA pool in unirradiated *P. pugio*.

The dicarboxylic amino acid, glutamic acid, occupies a central position in the metabolism of other amino acids, glycine, proline, and serine from the TCA cycle. In *P. pugio* it increased from the 5 ppt to the 20 ppt group. At the higher salinity level, a decrease in glutamic acid could be in response to greater production of the other amino acids, especially glycine which showed considerable increase at 35 ppt (Table 1).

Arginine, histidine, valine, cystine, leucine, methionine, isoleucine, phenylalanine and tyrosine are essential amino acids derived from the diet. All of these amino acids contributed little to maintaining intracellular osmotic integrity with the exception of arginine. Arginine contributed significantly, 12 to 17%, to the total intracellular free amino acid pool (Table 1). The concentrations of arginine are not greatly affected by salinity, but in the invertebrates, take on the role played by creatine phosphate in vertebrate muscle. It acts as a phosphagen and is important in muscular contraction in shrimp (Baldwin 1964).

Another amine, taurine, is found in substantial amounts in *P. pugio* intracellular FAA pool. Taurine is not an amino acid, but a sulfonated amine. On the basis of the experiments with arabinous-U-^{14}C, Gilles and Schoffeniels (1969b) proposed a metabolic pathway for taurine present

in crustaceans, a shunt of the TCA cycle. The role of taurine in marine invertebrates is that of an osmotic effector (Bedford 1969; Simpson, Allen, and Awapara 1959; Lange 1963). Taurine has been found in higher concentrations in marine invertebrates and correspondingly lacking in their fresh water counterparts, which indicates a direct relationship with osmoregulation (Awapara 1959). The metabolism and function of taurine in invertebrates has been extensively studied, but as of yet not fully understood. Taurine in *P. pugio* was highly variable in quanity and seemingly independent of the salinity level (Table 1).

Effects of Radiation and Salinity

Both time after irriadiation and salinity significantly affected the NPS concentrations in the muscle of grass shrimp (Figure 3). To test for interactions between radiation dose, time after irradiation, and salinity, a 3x3x5 analysis of variance (ANOVA) was calculated (Table 2). The analysis demonstrated that salinity interacted significantly with time after irradiation, and there was also a significant time-radiation dose interaction. Such interactions showed that the radiation produced a time dependent effect on NPS concentration.

In addition to looking at the NPS values as seen in the ANOVA, it is of interest to group the NPS data another way. By breaking down the data by salinity and radiation dose and looking at the variances, differences in the levels of irradiation were clearly shown (Table 3). The variance among the non-irradiated animals was much lower than at all three levels of irradiation with the variance greatest in the group of shrimp held at 35 ppt and irradiated at 1800 rads. These greater

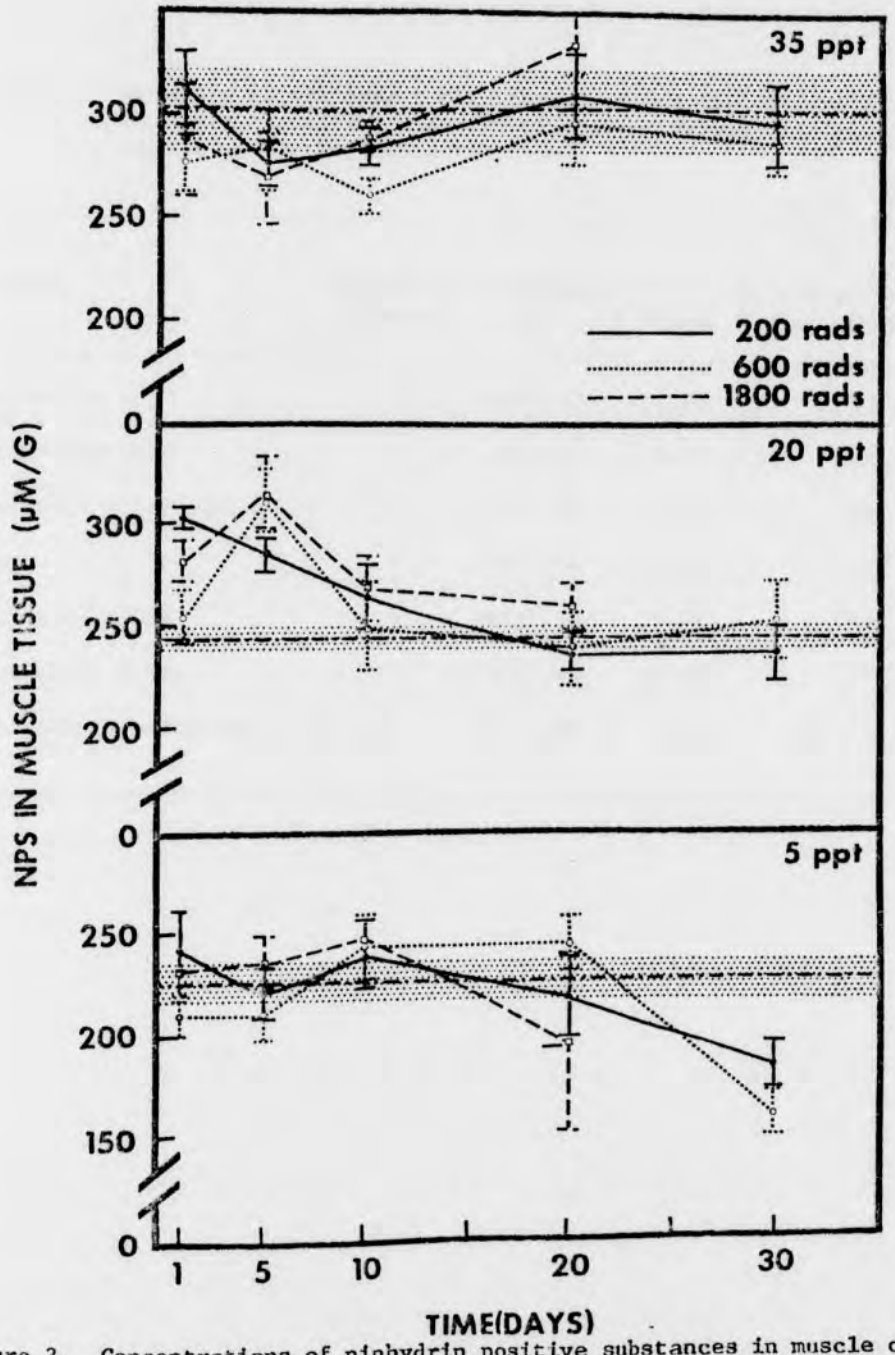

Figure 3. Concentrations of ninhydrin positive substances in muscle of irradiated *P. pugio*.. Shaded area is range of concentration in unirradiated *P. pugio*. Vertical bars indicate range of standard error.

Table 2.--Analysis of variance table (three way) of the effects of salinity, radiation dose and time after irradiation on shrimp muscle NPS concentrations.

Source	Degrees of Freedom	Partial SS	F Value	** = Signficant NS=Not Significant
Salinity	2	47185.43	21.28	**
Radiation Dose	2	2655.20	1.23	NS
Salinity X Radiation	4	9374.16	2.16	NS
Day	4	22707.78	5.24	**
Salinity X Day	8	29327.50	3.38	**
Radiation X Day	7	15918.41	2.10	**
Salinity X Radiation X Day	14	10438.89	0.69	NS

Table 3. Table of variances relating the salinity and the radiation dose to N.P.S. concentration in shrimp muscle.

		Radiation Dose (Rads)			
		0	200	600	1800
SALINITY LEVEL	5 ppt	371	1465	1539	967
	20 ppt	148	1045	1749	1532
	35 ppt	963	1610	925	1804

variances in concentration among the irradiated groups of animals occurred at all the salinities. All the levels of irradiation from 200 to 1800 rads showed fluctuations in concentrations. These higher variances regardless of salinity or dose level indicated an interference in the irradiated organisms ability to maintain the more constant levels of NPS seen in unirradiated *P. Pugio*.

Both radiation dose and time after irradiation interacted with salinity to cause changes in NPS levels in the shrimp muscle. Thirty days after irradiation, animals held at five ppt and exposed to 200 and 600 rads had decreased levels of NPS indicating possible osmoregulatory difficulty at low salinity (Figure 3). A similar trend is shown in the 1800 rads group after the 10th day. The decrease in NPS in the lower salinity group may be due to a change in cell permeability resulting in water influx, ion leakage or disruption of biosynthetic pathways of amino acids. NPS concentrations in shrimp at 20 ppt and irradiated with 600 and 1800 rads increased between the first and 5th day after irradiation, then decreased to control levels by Day 10. The increase in NPS may have resulted from stimulation of amino acid synthesis or changes in cell permeability after irradiation. The shrimp irradiated with 200 rads showed high NPS levels on the first day of sampling, but this leveled off in the later sampling days to a range of NPS values near the controls at 20 ppt. The animals at 35 ppt and irradiated with 200 and 600 rads showed relatively little change throughout the sampling period. However, those irradiated with 1800 rads showed increased concentrations in NPS on the 20th day following

irradiation. This increase may have been related to either intracellular osmoregulatory difficulties resulting in water loss or increase in synthesis. Also, the lower NPS values on the 5th day of sampling indicated possible metabolic difficulties. Otherwise, all other NPS values in the 35 ppt test group are near the mean for control animals at 35 ppt.

The effects of radiation on the individual free amino acids in the intracellular pool where both time and salinity dependent (Tables 4, 5, & 6). The individual free amino acids in *P. pugio* occurred in the same relative proportions in unirradiated and irradiated *P. pugio*, but some modifications were noted. The overall effects on the essential aminio acids, phenylalanine, tyrosine, leucine, isoleucine, methionine and valine were minimal. Diet more than likely influenced the concentrations as much as did irradiation, but the reduced food intake may have been the result of irradiation also. The non-essential amino acids, aspartic acid, serine, threonine, glutamic acid, proline, glycine, and alanine fluctuated in response to both salinity and time after irradiation.

Glycine and proline in unirradiated and irradiated *P. pugio* were the two primary contributors of the total free amino acid pool. At all three salinities both glycine and proline fluctuated widely throughout the experiment (Figure 4 and 5). In comparing these two amino acids, their respective concentrations showed somewhat inverse relationship which suggests compensation in maintaining the concentration of the free amino acid pool. This balancing effect was

Table 4. Concentrations of free amino acids in irradiated grass shrimp held at 5 ppt. Shrimp irradiated at the higher dose level died by the latter days of sampling. (Concentration expressed as μM amino acid/gram muscle tissue).

	Day after Irradiation														
	1			5			10			20			30		
	Irradiation Dose (rads)														
	200	600	1800	200	600	1800	200	600	1800	200	600	1800	200	600	1800
Lysine	1.3	2.0	1.9	1.9	1.7	2.8	1.8	2.3	1.9	1.9	1.0		2.2		
Histidine	1.1	0.9	0.8	0.8	0.6	2.2	1.2	1.9	1.1	1.6	0.9		--		
Arginine	38.5	34.0	43.0	42.6	37.7	34.0	34.1	43.6	29.7	36.4	39.2		32.5		
Taurine	24.3	17.6	32.8	21.1	31.0	13.8	24.6	20.1	16.5	28.2	15.0		31.8		
Aspartic Acid	0.4	0.6	0.7	0.5	1.1	1.1	0.6	0.9	1.3	3.4	0.6		0.8		
Threonine															
Serine	11.0	9.8	4.0	7.1	4.9	6.8	6.7	5.0	4.1	5.2	2.8		9.8		
Glutamic Acid	2.7	3.7	1.7	2.5	4.3	6.3	3.9	4.6	6.4	5.8	3.8		4.4		
Proline	24.8	37.0	36.0	24.7	20.7	27.1	45.4	13.2	11.5	29.6	27.5		35.5		
Glycine	74.7	65.7	76.1	98.3	92.5	106.0	101.2	117.1	117.3	84.3	140.8		100.4		
Alanine	15.1	25.9	25.1	16.3	12.9	21.5	11.4	15.9	13.0	18.6	12.3		11.1		
Cystine(Half)	tr	tr	tr												
Valine	1.6	1.4	tr	1.7	1.0	1.9	1.4	1.4	1.1	1.3	tr		2.4		
Methionine	1.3	0.8	0.1	0.	0.4	1.2	0.9	1.0	0.7	0.9	0.6		0.9		
Isoleucine	0.3	0.5	0.1	0.8	0.2	1.1	0.9	0.7	0.7	0.6	tr		1.4		
Leucine	1.2	1.3	tr	1.7	0.6	2.5	2.0	1.3	1.3	1.5	tr		2.8		
Tyrosine	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr		tr		
Phenylalanine	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr		tr		
Total free amino acids	208.3	201.2	222.3	222.7	209.7	228.3	236.1	233.4	206.3	219.6	244.8		235.7		

Table 5. Concentrations of free amino acids in irradiated grass shrimp held at 20 ppt. Shrimp irradiated at the higher dose level died by the latter days of sampling. (Concentration expressed as μM amino acid/gram muscle tissue).

	Day after Irridation													
	1			5			10			20			30	
	Irradiation Dose (rads)													
	200	600	1800	200	600	1800	200	600	1800	200	600	1800	200	600
Lysine	5.1	1.1	4.5	1.9	6.1	8.4	5.2	2.9	2.8	2.0	1.0	7.4	0.9	2.7
Histidine	2.9	1.6	2.8	0.6	2.8	2.5	2.3	1.9	1.4	1.4	0.4	1.2	tr	1.3
Arginine	48.7	53.1	44.3	48.6	53.8	50.6	46.2	44.1	43.9	38.5	35.3	34.0	31.2	33.1
Taurine	21.8	24.4	20.0	29.5	21.4	28.8	17.3	13.5	20.4	14.1	19.1	27.3	18.5	16.3
Aspartic Acid	1.4	0.6	1.3	0.7	1.1	1.7	1.5	1.1	2.5	0.9	1.4	2.7	1.9	1.6
Threonine														
Serine	15.6	5.8	19.2	4.2	20.4	19.7	15.7	15.2	7.5	10.0	6.5	1.7	6.3	9.8
Glutamic Acid	5.6	3.0	6.1	4.4	7.4	7.7		6.0	6.4	5.6	8.1	9.7	8.9	7.8
Proline	17.4	20.7	23.7	8.4	45.7	35.8	43.8	44.9	39.4	57.4	29.3	tr	29.7	34.8
Glycine	142.5	121.1	118.0	165.7	103.8	113.1	76.3	101.5	108.6	117.0	146.8	153.5	116.6	129.9
Alanine	24.0	16.4	17.4	16.5	27.9	32.9	21.1	14.7	22.7	16.0	15.7	9.0	12.5	19.2
Cystine(Half)											tr	tr	tr	tr
Valine	3.3	0.7	3.3	tr	4.7	5.1	3.6	2.5	1.9	tr	tr	tr	tr	1.9
Methionine	1.7	0.3	1.2	0.4	2.0	2.4	1.4	1.2	1.0	0.5	0.3	tr	tr	0.6
Isoleucine	2.1	0.2	1.7	0.4	3.2	3.8	1.1	0.6	0.6	tr	tr	tr	tr	tr
Leucine	4.4	0.5	4.5	1.9	7.2	8.5	3.0	2.3	2.0	tr	tr	tr	tr	1.1
Throsine	tr	tr	tr	tr	tr	tr	tr	tr	tr	--	tr	--	--	--
Phenylalanine	tr	tr	tr	tr	tr	tr	--	tr	--	--	--	--	--	--
Total free amino acids	296.4	249.5	267.8	316.7	307.4	321.0	238.5	212.5	261.0	258.1	263.9	246.7	227.1	244.8

Table 6. Concentrations of free amino acids in irradiated grass shrimp held at 35 ppt subjected to gamma irradiation. Shrimp irradiated at the higher dose level died by the later days of sampling. (Concentration expressed as μM amino acid/gram muscle tissue).

	Day after Irradiation												
	1			5			10			20		30	
	Irridation dose (rads)												
	200	600	1800	200	600	1800	200	600	1800	200	600	200	600
Lysine	1.0	1.3	0.7	1.7	1.4	2.5	0.8	1.1	1.0	2.9	2.1	1.0	0.8
Histidine	0.9	1.7	0.9	1.0	0.7	1.0	0.5	0.3	tr	1.4	0.8	0.5	0.4
Arginine	39.0	42.7	36.8	45.7	47.9	39.8	38.9	41.7	39.2	38.2	31.7	39.6	35.8
Taurine	11.3	14.6	19.6	11.7	18.7	16.3	10.3	19.9	16.9	9.9	8.9	12.5	7.9
Aspartic Acid	1.3	1.1	1.1	0.9	1.0	0.3	0.5	1.0	0.4	0.8	1.2	2.2	0.1
Threonone													
Serine	9.4	14.0	5.7	13.5	8.2	13.9	9.0	7.2	3.9	18.5	4.0	3.0	1.7
Gultamic Acid	4.2	3.4	3.2	2.2	2.5	2.7	2.4	3.6	3.6	2.2	4.7	7.7	4.9
Proline	25.9	52.0	38.5	70.4	60.0	66.0	48.8	60.2	48.5	68.5	52.3	32.5	19.7
Glycine	179.2	125.9	260.6	119.9	126.2	120.5	146.2	121.4	144.3	133.0	140.5	155.3	186.6
Alanine	19.2	23.9	17.0	26.0	31.9	25.8	23.1	23.8	19.6	26.9	18.1	21.1	9.1
Cystine(Half)	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr			
Valine	tr	1.7	tr		1.0	1.9	tr	tr	tr	3.3	2.2	1.1	0.3
Methionine	0.4	0.3	tr	0.6	0.3	0.5	tr	0.2	tr		1.2	0.5	0.5
Isoleucine	tr	0.3	tr	0.8	0.4	1.2	tr	tr	tr	1.8	1.3	0.4	--
Leucine	0.9	1.1	tr	2.0	1.1	2.4	tr	0.3	0.4	4.6	2.8	0.9	--
Tyrosine	--	tr	tr	tr	tr	tr	--	--	tr	--	--	--	--
Phenylalanine		tr	tr	--	tr	tr	--	--	tr	--	--	--	--
Total free amino acids	292.9	282.4	284.2	296.4	301.0	291.9	280.6	280.9	277.9	311.7	274.7	279.5	268.0

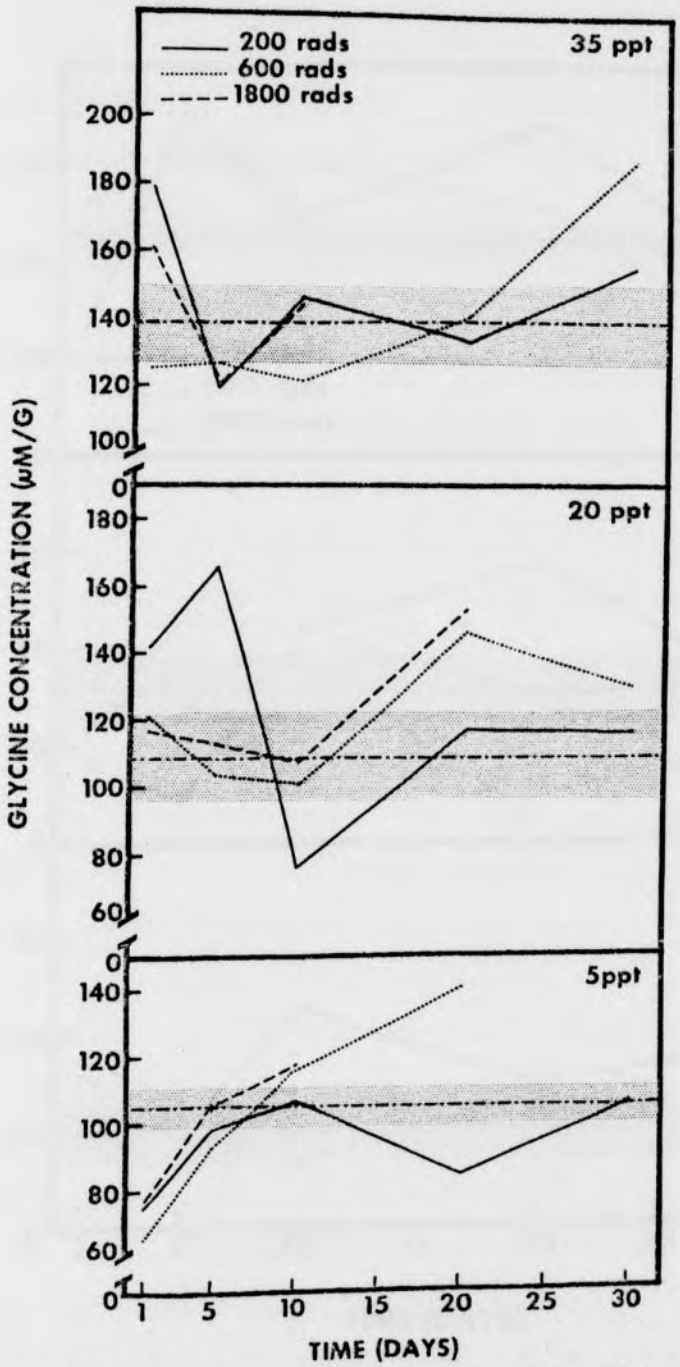

Figure 4. Concentrations of glycine in muscle of irradiated *P. pugio*. Shaded area indicates range of concentration in unirradiated *P. pugio*.

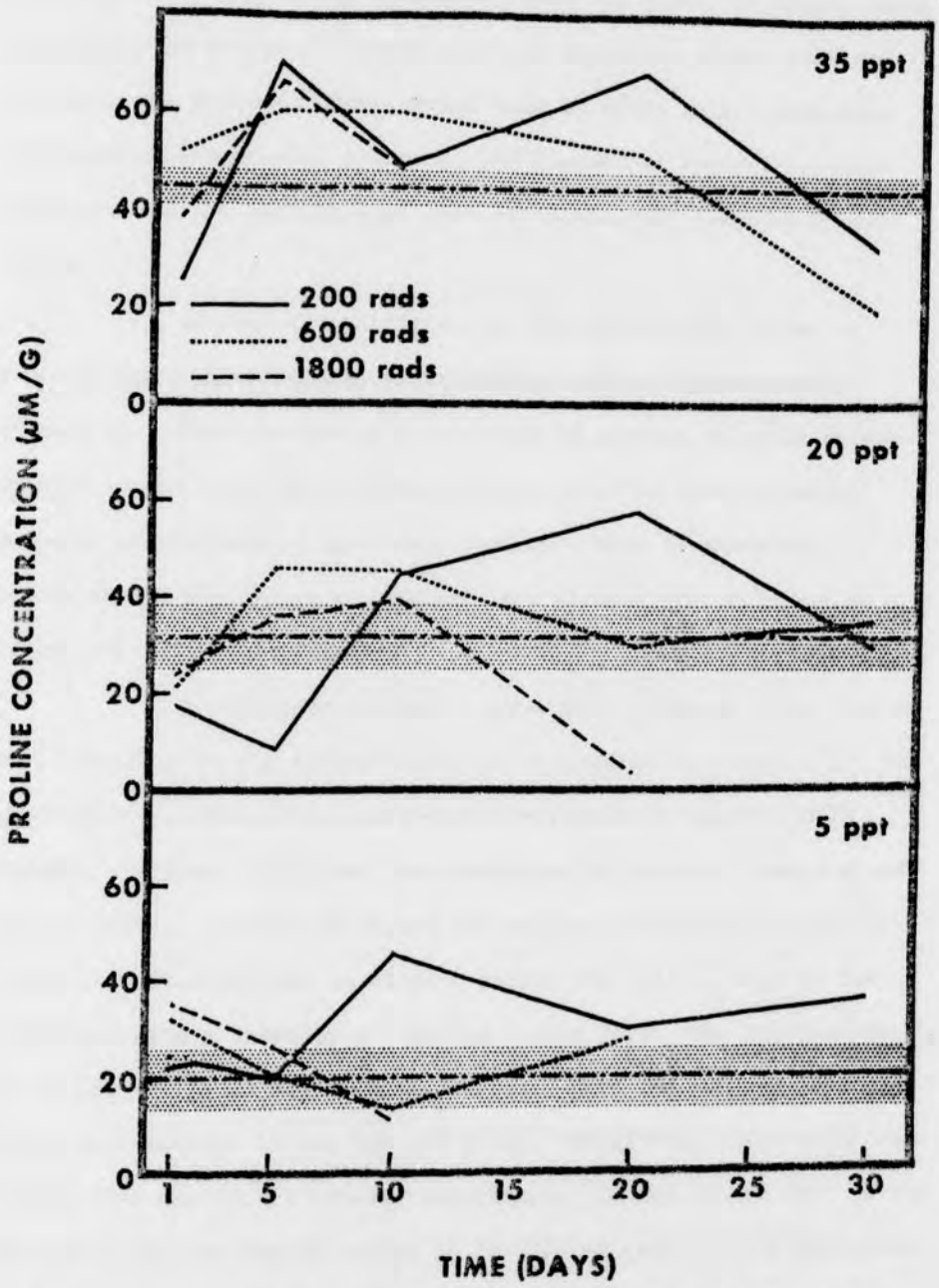

Figure 5. Concentrations of proline in muscle of irradiated *P. pugio*. Shaded area indicates range of concentration in unirradiated *P. pugio*.

most obvious in the shrimp irradiated with 200 rads. At higher doses somatic damage and cell leakage may be a factor to either mask or eliminate the process. Even though both of these amino acids have independent biosynthetic pathways, the individual control of their sysnthesis may be through some type of interacting feedback control system.

Serine concentrations in the muscle also tended to counterbalance fluctuations in irradiated glycine concentration (Figure 6). Since serine is a precursor of glycine it seems reasonable to assume that their concentrations could be inter-related. However, the pathway of synthesis just described is mammalian or microbial and the crustacean pathway for glycine systhesis may be different and not involve serine.

Another non-essential amino acid, alanine, also fluctuated erraticly in the initial days after irradiation (Figure 7). Its biosynthetic pathways include β-decarboxylation of aspartic acid (Florkin and Mason 1962) and transamination of pyruvate (Awapara and Simpson 1967). Irradiated shrimp in the five ppt and 35 ppt groups showed wide fluctuations in alanine during the initial days of the experiment with a leveling off in the latter days. The fluctuations in the 20 ppt shrimp on Day 5 showed increase with the 600 and 1800 rad groups and decrease in the 200 rad group. Total free amino acids were high on this day in all irradiation groups. In the latter days of the experiment alanine concentration in irradiated grass shrimp approached the quantities of alanine in unirradiated *P. pugio*. The greatest

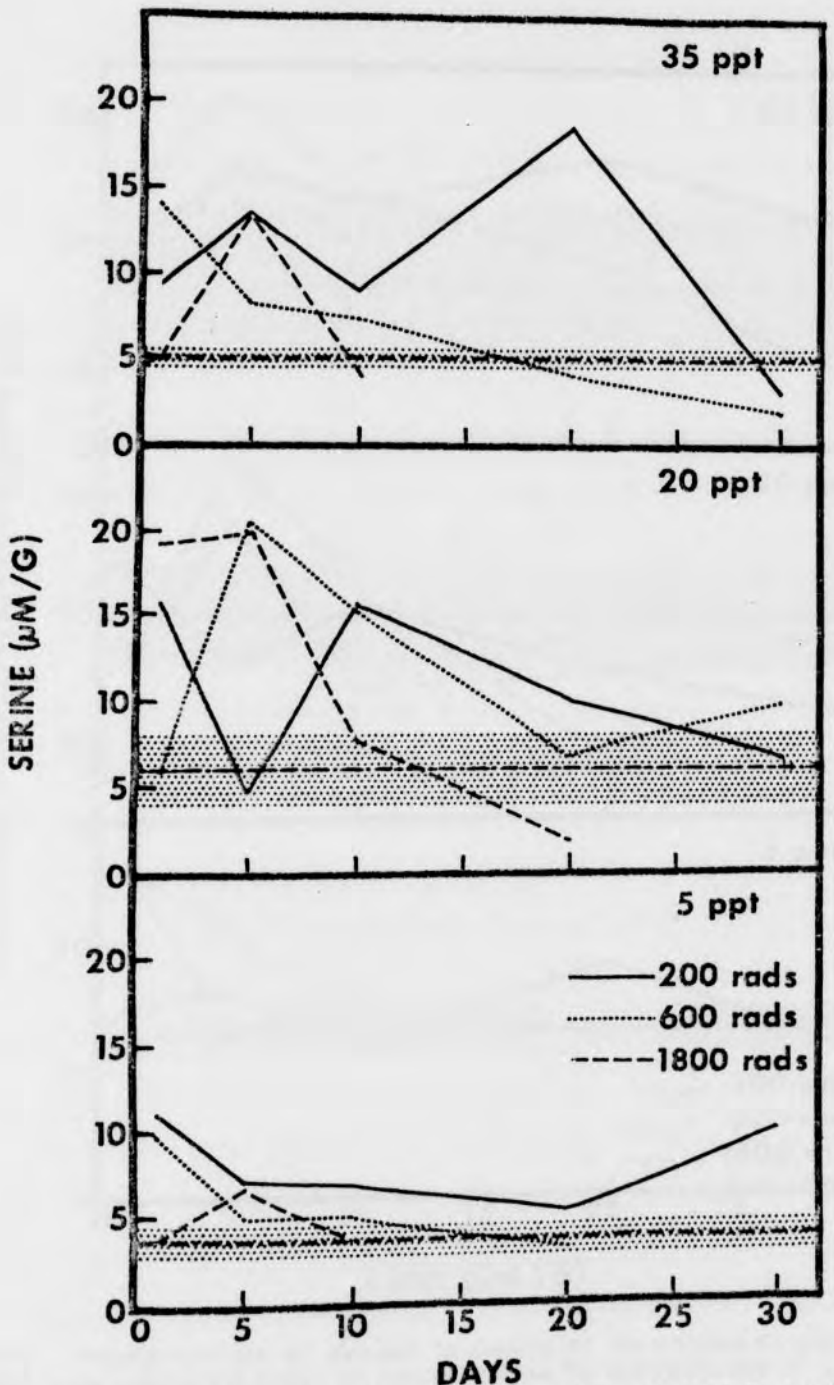

Figure 6. Concentrations of serine in muscle of irradiated *P. pugio*. Shaded area indicates range of concentration in unirradiated *P. pugio*.

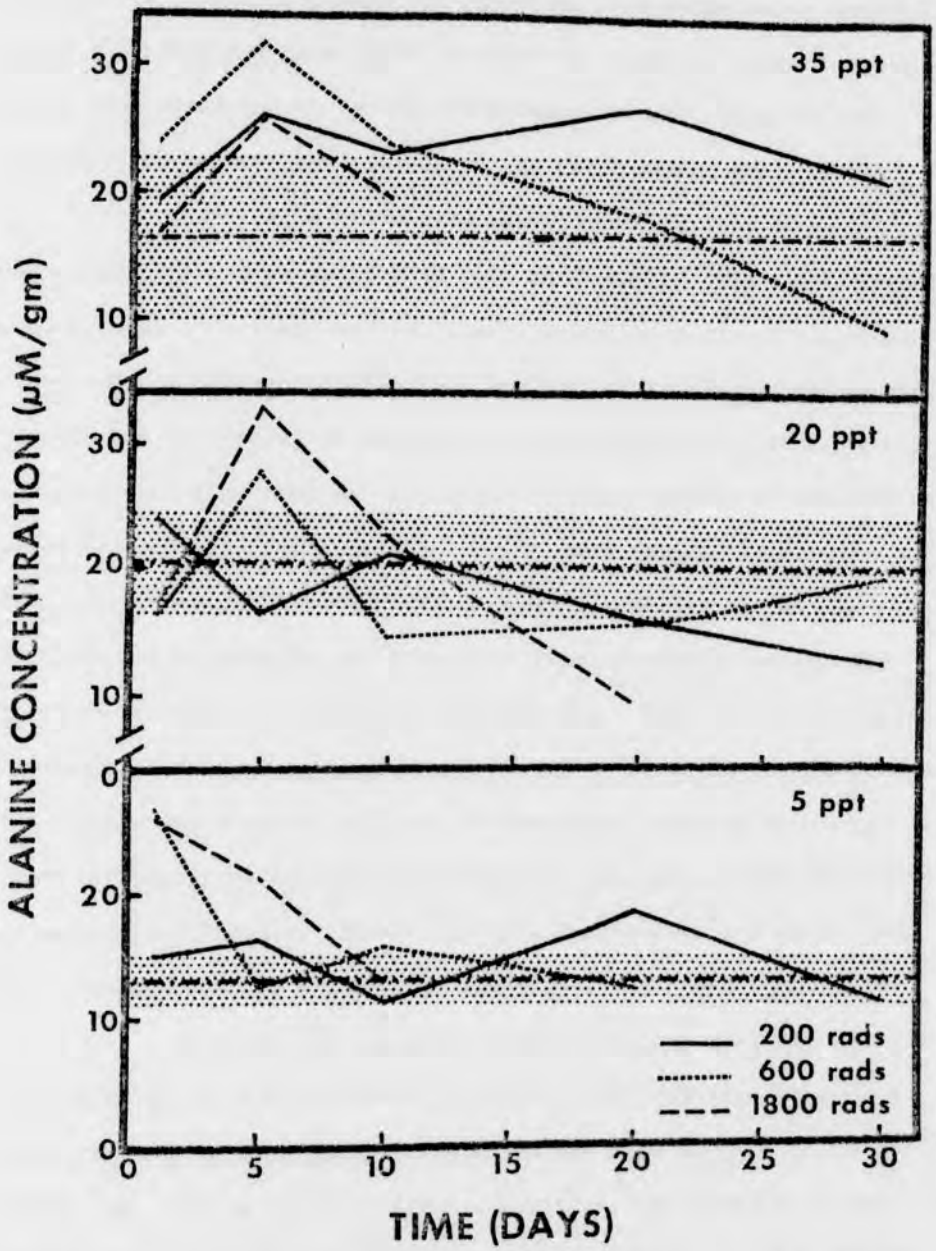

Figure 7. Concentrations of alanine in muscle of irradiated *P. pugio*. Shaded area indicates range of concentration in unirradiated *P. pugio*.

fluctuations in all the above non-essential free amino acids occurred within the first ten days after irradiation exposure. Later samples showed less variation in amount and concentrations, closer to the individual free amino acid levels of the unirradiated grass shrimp.

Taurine, a sulfonated amine, makes up from 5% to 15% of the intracellular free amino acid pool in *P. pugio*. The concentrations of taurine were variable and not always described a direct relationship to the ambient salinity (Table 1). The role of taurine as an osmotic affector may be related to radiation damage (Figure 8). Since it has been suggested that taurine may be selectively retained or released as needed for osmoregulation (Bedford 1955), in *P. pugio* there may be a either selective retention of taurine when metabolism of the non-essential amino acids is inhibited or a taurine release occurs when FAA's are available for active osmoregulation. That is, the retention or release of taurine may be related to (1) difficulties in the production of free amino acids, (2) use of some amino acids as an energy source for repair of irradiation damage or, (3) amino acids being used for protein manufacture. These effects on metabolism are subtle and may be linked to other biochemical activities not investigated here.

Arginine, an essential amino acid, occurred in significant quantities in the muscle of *P. pugio* in all salinity groups and it was affected by time after irradiation and dose (Figure 9). Since arginine was present in significant quantities, its function is open to question. In working with mantle and muscle tissue in the scallop, *Argopectin* Engel (1976, unpublished data) found arginine in the muscle

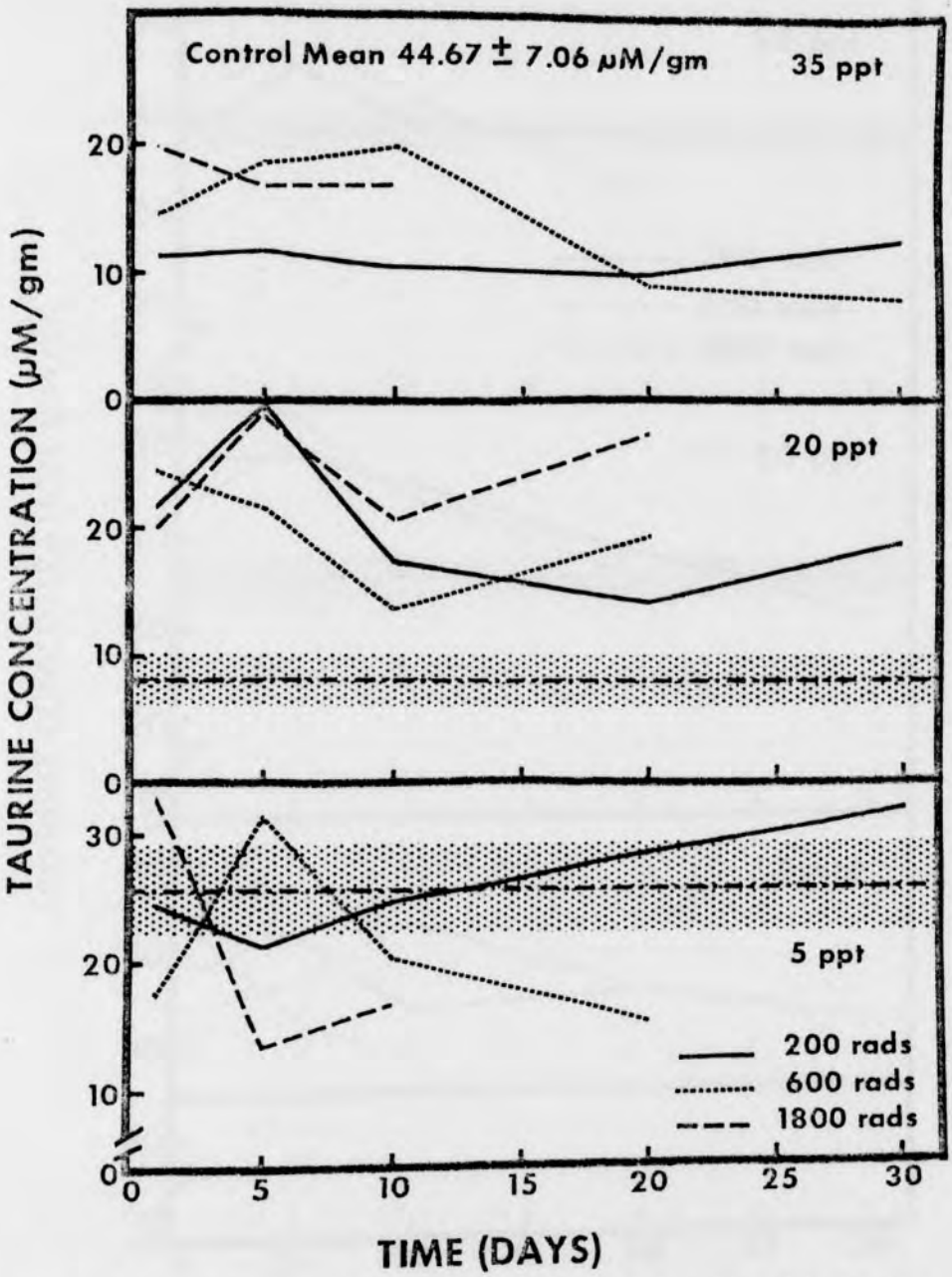

Figure 8. Concentrations of taurine in muscle of irradiated *P. pugio*. Shaded area indicates range of concentration in unirradiated *P. pugio*.

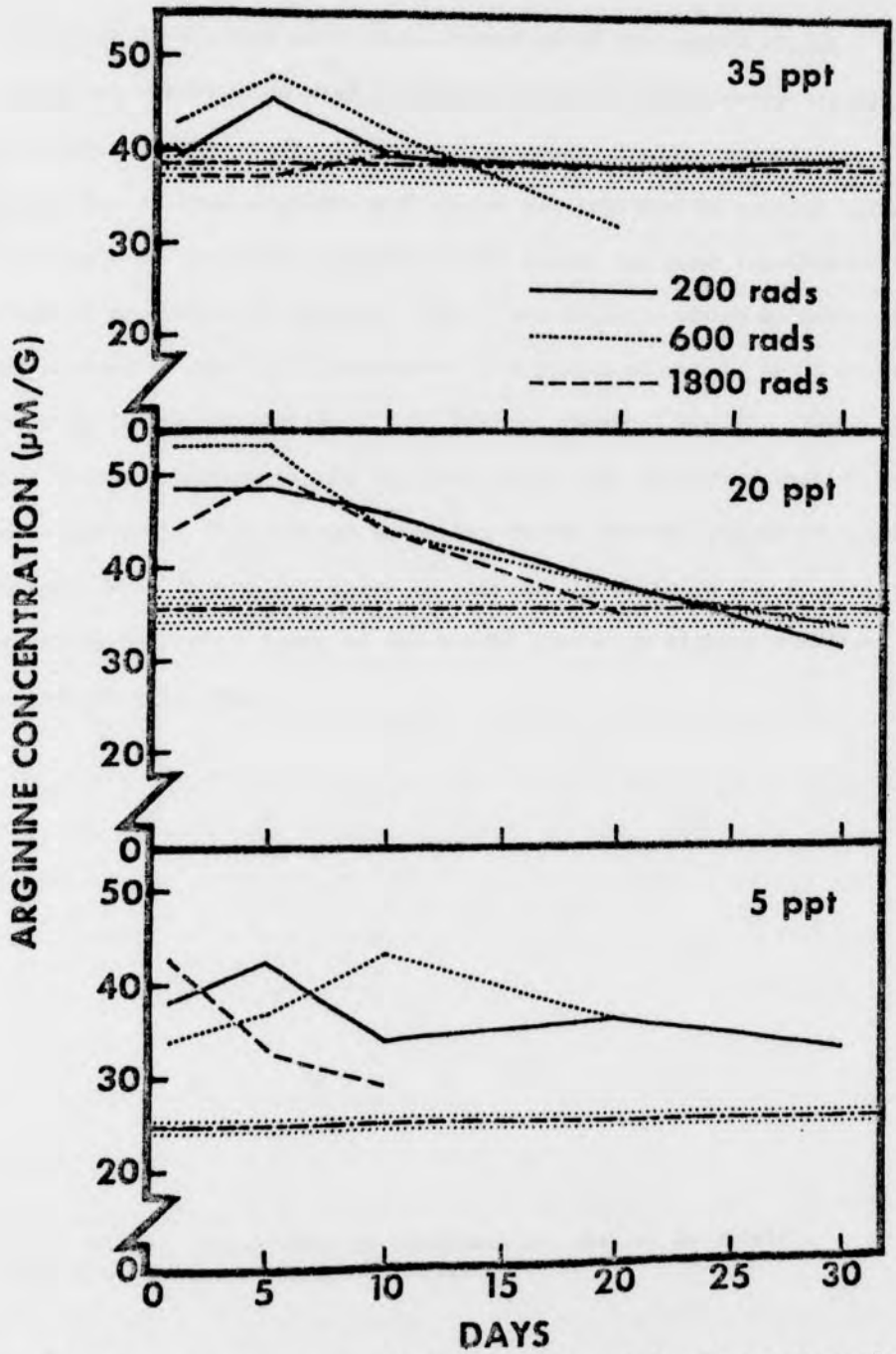

Figure 9. Concentrations of arginine in muscle of irradiated *P. pugio*. Shaded area indicates range of concentration in unirradiated *P. pugio*.

approximately 6 times more concentrated as in the mantle tissue, suggesting correlation with the greater need of this energy supplying phosphagen in the muscle tissue. Crustaceans lack a significant production of free arginine and almost all arginine is present intracellularly as arginine phosphate which serves the same function as creatine phosphate in mammals. Thus, the arginine which we detect is the product of the acid hydrolysis of arginine phosphate by 5% tichloroacetic during extraction (S. H. Bishop, personal com.[1]). The radiation induced changes in the arginine levels may therefore reflect the mobilization of this energy releasing system for the repair of cellular damage. This damage may cause an increase in arginine due to an increase in metabolic level of the muscle tissue or altered membrane permeability to ions.

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CONCLUSIONS

Radiation induced changes in both the intracellular total and individual free amino acid pools was time-dependent and highly variable. The physiological and metabolic condition of the individual, both at the time of irradiation and when the particular sample was taken, may be a source of variation within and between individuals. At all three salinities, irradiated *P. pugio* showed trends of fluctuation above and below the NPS values of unirradiated shrimp. Dehydration or hydration of the shrimp following irradiation did not account for the variability in the totals. All the amino acids did not increase or decrease as a unit, but instead some amino acids acted to compensate for other amino acids which may be unusually high or low, thus maintaining osmotic balance depending on the external osmotic pressure. Apparent compensation was most prevalent among those amino acids associated with glycolysis and the TCA cycle, more notably glycine, proline, alanine, and glutamic acid. This relative change may result from several factors: (1) possible re-entry of individual amino acids into the TCA cycle for use as an energy source; (2) metabolic compensation stimulated by varying quantities of intermediates in the TCA cycle; (3) damage to synthetic pathways for specific amino acids compensated for by the production of other amino acids through some type of feedback control. Interference in the metabolic pathways of some amino acids may result in the subsequently greater concentration of other non-essential amino acids. Alanine, glycine, serine, proline, and glutamic acid concentrations

fluctuated following irradiation with a decrease in one or several of these amino acids leading to an increase in others which usually resulted in a balancing out in the total free amino acid pool. The use of free amino acids as an energy source is reasonable since most are synthesized via transamination from glycolytic and TCA cycle intermediates. For example, serine, alanine, cysteine or threonine may be converted to pyruvate, and proline, arginine, histidine, or glutamic acid may enter the TCA cycle by deamination and conversion to α-ketoglutaric acid. The use of amino acids as an energy reserve may also result from reduced feeding caused by the irradiation. Also, damage to selective transport systems may lead to erratic fluctuations in only a few amino acids, whereas membrane damage would affect concentrations of all the amino acids equally.

Another factor which may relate to variation is the effects of sampling. The combination of sampling the test populations, and deaths may select out the hardiest individuals. For example, the irradiated animals at all the salinity groups held until the latter days of the experiment had muscle NPS levels about the same as in non-irradiated *P. pugio* although there was the greatest mortality at the highest dose rates. The recovery may have been either the metabolic adjustment with time after irradiation, or a selection of more resistent shrimp. In effect, the sampling of a population after irradiation indicated the possibility of (1) irradiation damage repair, or (2) a survival of individuals which sustained less initial damage.

The purpose of the investigation was to test the hypothesis that irradiation affects the osmoregulatory ability of *P. pugio* and

therefore may contribute to the radiosensitivity of this organism. The results indicate that the concentrations of intracellular free amino acids vary directly with salinity. The intracellular amino acid pool in irradiated *P. pugio* fluctuated both with radiation dose and time after irradiation and suggested a synergistic effect between irradiation and salinity to disrupt metabolism thus interfering with one phase of osmoregulation.

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